

**Fe and Zn K-edge XAFS Investigation of the Relationship Between the Ligand Structure and Enzymatic Activity of Endothelial Nitric Oxide Synthase**

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Beamline(s): X9B

**Introduction:** Ultra dilute Fe and Zn K-edge XAFS experiments have been performed on endothelial nitric oxide synthase (NOS) in frozen solution. This enzyme is found in the endothelial lining of blood vessels, and synthesises nitric oxide NO. This small volatile molecule is needed for regulation of the diameter of blood vessels, and, as such, is crucial for the regulation of blood pressure. All major risk factors for atherosclerosis, such as smoking, dyslipidemia, diabetes, hypertension and hyperhomocysteinemia, have been shown to correlate with in vivo deficiency of NO. NOS is only active in dimeric state and requires a special pterin (BH<sub>4</sub>) as a cofactor. In absence of BH<sub>4</sub>, NOS is known to produce large quantities of superoxide radicals, which act as strongly atherosclerotic agents. In endothelial NOS, both NO and superoxide are known to be produced at the heme domain of the enzyme. Therefore, the problem of the role of BH<sub>4</sub> in enzymatic activity of NOS is central to the development of atherosclerosis.

**Methods and Materials:** The sample preparation required for our experiments is highly involved since NOS preparations must be obtained in absence of BH<sub>4</sub> free NOS was synthesized by genetic transfection of adenoviruses with DNA sequences for NOS, bringing these to expression and harvesting and purifying the NOS material. The NOS enzyme was investigated in two shifts at Beamline X9B.

**Results:** Although the EXAFS analysis of the Zn-spectra are not completed, a number of important conclusions can already be drawn at this stage

1. The Fe-EXAFS data have shown that the liganding structure of NOS heme closely resembles that of Cytochrome P450 (a well investigated heme enzyme which has been been crystallized). In particular, the presence and distance of a characteristic sulphur ligand in axial position could be confirmed.
2. It was verified that each NOS dimer contains roughly two iron atoms and a single zinc atom. The ligand structure of the Zn is very similar to that found in the crystallized dimers of the heme-subunits. It shows that, in fully functional NOS dimers, the individual monomers are oriented head to head (i.e. that the heme domains are adjacent).
3. Incubation of NOS with BH<sub>4</sub> does not induce observable changes in the ligand structure of the heme. This observation is compatible with the fact that the binding site for BH<sub>4</sub> has considerable distance to the heme (as shown by crystallographic data from the heme-dimer).
4. We have detected that incubation with BH<sub>4</sub> induces a small (ca 0.6 eV) upward shift in the edge position of the heme iron. This result is very exciting as it is the first indication that BH<sub>4</sub>, though far away, affects the charge state and redox properties of the heme group.
5. The quality of the data was so good that pre-edge structure of the Fe K-edge could be resolved. The analysis is in progress.

**Conclusions:** Our data lend strong support to the supposition that the crystallographic data obtained on dimers of the heme subtractions are representative of the situation of the complete NOS dimers in working state as well. We are particularly excited about the observed effect of BH<sub>4</sub> on the edge position of the heme. Though this observation has not been understood yet, we hope that it will shed light on the mechanism through which the cofactor BH<sub>4</sub> can make NOS to synthesis NO rather than highly noxious superoxide.